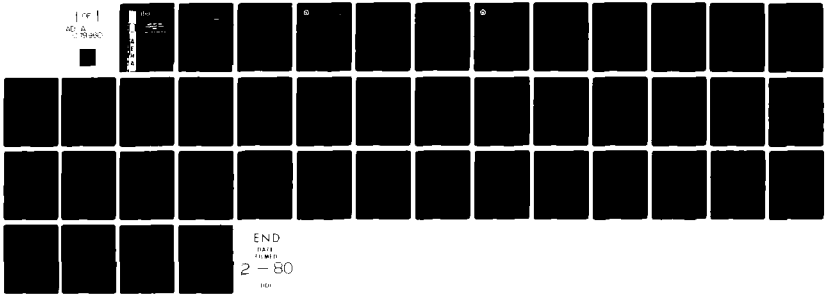
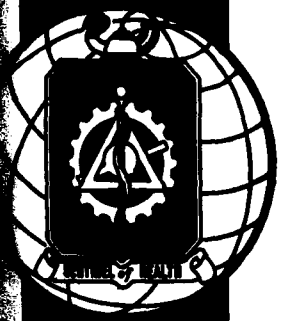
AD-A079 960 ARMY ENVIRONMENTAL HYGIENE AGENCY ABERDEEN PROVING GR--ETC F/G 6/20
INHALATION TOXICITIES OF N,N-DIETHYL-META-TOLUAMIDE (M-DET), JA--ETC(U)
JAN 80 J A MACKO, J D BERGMANN
UNCLASSIFIED USAEHA-75-51-0034-80 NL



END
DATE FILMED
2-80
DDC

LEVEL II

12



UNITED STATES ARMY ENVIRONMENTAL HYGIENE AGENCY

ABERDEEN PROVING GROUND, MD 21010

PHASE 4
INHALATION TOXICITIES OF N,N-DIETHYL-META-TOLUAMIDE (M-DET)
STUDY NO. 75-51-0034-80
JANUARY-MAY 1979

Approved for public release; distribution unlimited.

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE	READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER: 75-51-0034-80	2. GOVT ACCESSION NO. / 3. RECIPIENT'S CATALOG NUMBER: 9
4. TITLE (and Subtitle): Phase 4 Inhalation Toxicities of N,N-Diethyl-Meta-Toluamide (M-Det), [redacted] January-May 1979	5. TYPE OF REPORT & PERIOD COVERED: Special Study on Phase 4 [redacted]
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): JOSEPH A. MACKO, JEFFREY D. BERGMANN	8. CONTRACT OR GRANT NUMBER(s): 11 21 Jan 80
9. PERFORMING ORGANIZATION NAME AND ADDRESS: US Army Environmental Hygiene Agency, Aberdeen Proving Ground, Maryland 21010	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: 12 44
11. CONTROLLING OFFICE NAME AND ADDRESS: Commander, US Army Health Services Command, Fort Sam Houston, TX 78234	12. REPORT DATE
	13. NUMBER OF PAGES: 38
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)	15. SECURITY CLASS. (of this report): Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release;distribution unlimited.

14 USAEHA-75-51-0034-80

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

N,N-Diethyl-M-Toluamide	Insect Repellent
M-Det	Aerosol
Inhalation	Saturated Vapor
Acute	
Subchronic	

100 c

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

No toxic signs were seen in the six male rats exposed to saturated vapor of m-Det at room temperature for an 8-hour exposure period. Transient hyperemia was seen in rats during the 100 m-Det saturated vapor exposure. The acute aerosol LC_{50} for rats was 5950 mg/m³.

Transient toxic signs were seen in the rats and dogs exposed to m-Det during the 13-week subchronic inhalation study. Toxic signs ranged from red exudate

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 68 IS OBSOLETE

Unclassified 038 150

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

Item 20. Abstract (continued).

around the eyes and noses of the rats exposed to 1500 mg/m^3 of m-Det to periodic vomiting of dogs exposed to 750 and 1500 mg/m^3 of the test compound. No gross or histological lesions were attributable to the inhalation of m-Det aerosol. M-Det may have induced spermhead abnormalities in rats under the condition of the subchronic inhalation exposure, although the observed effects were small.

It is concluded that inhalation of m-Det insect repellent at the 750 mg/m^3 level and below presents little acute inhalation hazard to man. Concentrations above this level may cause transitory eye and respiratory irritation. M-Det may induce spermhead abnormalities at the high concentration (1500 mg/m^3) tested, although the observed effects were small. Behavioral changes were evidenced at all exposure levels, but their significance to human health is unknown.

DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

Mr. Macko/lr/AUTOVON
584-3980

HSE-LT/WP

21 JAN 1980

SUBJECT: Phase 4, Inhalation Toxicities of N,N-Diethyl-Meta-Toluamide (M-Det), Study No. 75-51-0034-80, January-May 1979

Executive Secretary
Armed Forces Pest Control Board
Forest Glen Section
Walter Reed Army Medical Center
Washington, DC 20012

A summary of results and conclusions of the inclosed report follows:

a. No toxic signs were seen in the six male rats exposed to saturated vapor of m-Det at room temperature for an 8-hour exposure period. Transient hyperemia was seen in rats during the 100° m-Det saturated vapor exposure. The acute aerosol LC_{50} for rats was 5950 mg/m^3.

b. Transient toxic signs were seen in the rats and dogs exposed to m-Det during the 13-week subchronic inhalation study. Toxic signs ranged from red exudate around the eyes and noses of the rats exposed to 1500 mg/m^3 of m-Det to periodic vomiting of dogs exposed to 750 and 1500 mg/m^3 of the test compound. No gross or histological lesions were attributable to the inhalation of m-Det aerosol. M-Det may have induced spermhead abnormalities in rats under the condition of the subchronic inhalation exposure, although the observed effects were small.

c. It is concluded that inhalation of m-Det insect repellent at the 750 mg/m^3 level and below presents little acute inhalation hazard to man. Concentrations above this level may cause transitory eye and respiratory irritation. M-Det may induce spermhead abnormalities at the high concentration (1500 mg/m^3) tested, although the observed effects were small. Behavioral changes were evidenced at all exposure levels, but their significance to human health is unknown.

FOR THE COMMANDER:

Brendan E. Joyce

BRENDAN E. JOYCE, Ph.D.
COL, MSC
Director, Laboratory Services

1 Incl
as

CF:
HQDA (DASG-PSP)
Cdr, HSC (HSPA-P)
Dir, Advisory Cen on Tox, NRC
Supt, AHS (HSA-IPM)
USDA, ARS-Southern Region
USDA, ARS (Dr. T. McGovern)

Study No. 75-51-0034-80, Jan-May 79

CONTENTS

Study No. 75-51-0034-80, Jan-May 79

DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

HSE-LT/WP

INHALATION TOXICITIES OF N,N-DIETHYL-META-TOLUAMIDE (M-DET)
STUDY NO. 75-51-0034-80*†
JANUARY-MAY 1979

1. AUTHORITY.

a. Memorandum of Understanding between the US Army Environmental Hygiene Agency; the US Army Health Services Command; the Department of the Army, Office of The Surgeon General; the Armed Forces Pest Control Board; and the US Department of Agriculture, Agricultural Research, Science and Education Administrations; titled Coordination of Biological and Toxicological Testing of Pesticides, effective 23 January 1979.

b. Letter, Armed Forces Pest Control Board, 17 March 1977, subject: Reregistration Data for N,N-Diethyltoluamide Repellent.

2. REFERENCES. See Appendix A for a listing of references and Appendix B for infrared spectrum of N,N-Diethyl-Meta-Toluamide.

3. ABBREVIATIONS. A list of specialized terms and abbreviations are listed in Appendix C.

4. PURPOSE. The purpose of this study was to determine the inhalation toxicity in rats and dogs exposed to vapors and aerosols of m-Det. This information will be used to predict possible health hazards to humans who receive single or repeated inhalation exposures to the insect repellent, m-Det.

---

* In conducting the studies described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," US Department of Health, Education, and Welfare Publication No. (NIH) 78-23, revised 1978.
† The experiments reported herein were performed in animal facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Use of trademarked names does not imply endorsement by the US Army, but is intended only to assist in identification of a specific product.

5. BACKGROUND. M-Det is the Army's standard insect repellent. It is intended for use as a solution in ethanol and as an aerosol for direct application to the skin and/or clothing. The original laboratory data developed by Ambrose (reference 1, Appendix A) are no longer available, which necessitates further studies to determine the inhalation health effects of technical grade m-Det.

6. PROCEDURES. Two types of inhalation studies were conducted: (1) exposure to air saturated with vapor; and (2) exposure to an aerosol (acute and subchronic).

a. Test Material. The test material used during these studies was m-Det with a minimum meta isomer content of 95 percent and 5 percent maximum of other isomers. The material (Lot No. 7141) was manufactured by Hardwicke Chemical Company, Elgin, SC 29045, and packaged for McLaughlin Gormly King Company, 8810 Tenth Avenue North, Minneapolis, MN 55427.

b. Animals.

(1) Sprague-Dawley albino male and female rats obtained from USAEHA's colony were used in these studies. The rats were numbered sequentially by the Division Veterinarian and identified by toe clipping. They were housed in groups of five, with food and water offered ad libitum between exposures. The laboratory diet for the rats was Formulab Chow® No. 5008.

(2) Purebred Beagle dogs, 1 year of age, purchased from Laboratory Research Enterprises, Kalamazoo, MI 49009, were used during the subchronic inhalation exposure. Dogs were numbered sequentially by the Division Veterinarian and identified by ear tatoo. Exposure groups were housed individually, with food and water offered ad libitum. The laboratory diet for the dogs was Purina Lab Canine Diet, Ralston Purina Company, St Louis, MO 63188.

(3) All animals were deprived of food and water during exposure periods.

(4) All cages and inhalation chambers were color coded for each exposure and control group of animals.

● Formulab Chow No. 5008 is a registered trademark of Ralston Purina Company, St Louis, MO 63188.

c. Saturated Vapor Exposure. Three groups of six male rats (starting weights = 132 ±8.35 gm) were exposed to air saturated with m-Det at 100°C and 26°C, or to room air with no m-Det. Incoming air was bubbled through m-Det contained in a glass gas-washing bottle with a fused fritted cylinder at a flow rate of 2 liters per minute before passing into the inhalation chamber. The chambers were 9-liter glass desiccators with six individually screened compartments. The gas-washing bottle in the 100°C exposure was immersed in an oil bath and heated. The room and oil-bath temperatures were monitored throughout the 8-hour exposure. The weight of m-Det dispersed during the exposures was measured to determine the nominal concentration of each exposure.

d. Acute Aerosol Inhalation.

(1) Acute aerosol inhalation studies were performed to determine the lethal aerosol concentration (LC_{50}) for 50 percent of the male and female rats. Ten rats per sex (107 ±15 gm) were used at each concentration tested, i.e., time-weighted average concentration of 3700, 4260, 5185, 5450, 5480, and 5950 mg/m^3. A control group composed of the same number of animals of each sex was also included for each exposure group. Rats were individually caged and placed in a Wahmann 225 L, dynamic airflow exposure chamber. The test compound was dispersed into the chambers for 4 hours using a Collison Nebulizer (BEI, Inc. of Waltham, MA). The rate of airflow through the chamber as well as temperature were monitored during the exposures. The actual concentration of m-Det in the animals' breathing zones was measured four times during each exposure (0.5, 1, 2, and 3 hours into the exposure). Chamber air was pulled through a glass fiber filter at approximately 2 liters per minute for 5 minutes. The filter contents were extracted with hexane and then analyzed by gas chromatography. A Shimadzu GC-MINI-1 gas chromatograph with a one-eighth inch stainless steel, 1.5 meter, 10 percent SP2100 on 80/100 Supelcoport (Supelco, Inc.) column was used at 200°C. A particle Count Median Diameter measurement of the dispersed compound was attempted using a Teflon®-coated slide introduced into the chamber 30 minutes after onset of the 4-hour exposure. The slide was attached to the end of an 18-inch ruler and introduced 2 inches deep through a rear port located in the center of the chamber and held for 1 minute. However, particle-sizing determinations were impossible due to the speed of evaporation of the Jet from the Teflon-coated slides.

(2) Rats were observed during the exposure and for 14 days post exposure. Body weights were determined on the day of exposure and days 1, 3, 7, and 14 days post exposure. At the conclusion of each 14-day observation period, the surviving animals were sacrificed by decapitation and internal organs examined for gross abnormalities. Tissues exhibiting gross abnormalities were preserved for future histopathologic examination.

® Teflon is a registered trademark of E. I. DuPont deNemours & Co., Inc. Wilmington, DE.

(3) Acute behavioral evaluation used identical techniques and rat groups, but the concentrations were 4100, 2900, and 2300 mg/m^3. Just after exposure, each rat was exposed to a series of tests which measured balance, endurance, activity, tactile sensitivity, learning, and recall. These tests are described in a separate report (reference 2, Appendix A).

e. Subchronic Aerosol Inhalation.

(1) This study was done to determine the inhalation toxicity in rats and dogs exposed to aerosols of m-Det during a 6-hour per day, 5-day per week, 13-week study. Acute aerosol inhalation work indicated that the 4-hour LC_{50} is 5950 mg/m^3. Based on this finding, the three aerosol concentrations to be used in this 6-hour study were 1500 mg/m^3 (1/4 LC_{50}), 750 mg/m^3 (1/8 LC_{50}), and 250 mg/m^3 (1/24 LC_{50}). The test material (see paragraph 6a) was administered as an aerosol using a Collison Nebulizer by whole-body exposures of rats and dogs in 2000-liter chambers (Washington Technology Associates, Inc., Rockville, MD) and two 1000-liter chambers (Howell Laboratories, Inc., Bridgeton, ME 04009). The animals (see paragraph 6b) were selected at random after breeding and were assigned to the groups listed in Table 1.

TABLE 1. DOGS AND RATS IN SUBCHRONIC AEROSOL STUDY

		Rats		Dogs			
No.	Group Color Code	Male	Female	Male	Female	Conc. Level	Fraction of Rat LC_{50}
1	White	(#251-270) 20	(#331-350) 20	(#53-54) 2	(#63-64) 2	Room Air	-
2	Red	(#271-290) 20	(#351-370) 20	(#47-48) 2	(#65-66) 2	1500 mg/m^3	1/4
3	Blue	(#291-310) 20	(#371-390) 20	(#49-50) 2	(#67-68) 2	750 mg/m^3	1/8
4	Green	(#311-330) 20	(#391-410) 20	(#51-52) 2	(#69-70) 2	250 mg/m^3	1/24

Chamber air was sampled three times (1, 3, and 5 hours into exposure) daily to determine actual concentrations. Samples were analyzed by gas chromatography as in the acute study (see paragraph 6d). Microscopic examination of the particles for Count Median Diameter determination was impossible due to the speed of particle evaporation from the Teflon-coated glass sample slides, as was demonstrated during the acute inhalation study. Control animals were caged in two 1000-liter chambers (Howell Laboratories,

Inc., Bridgeton, ME 04009). The animals were exposed daily for 6 hours, 5 days per week, for 13 weeks. The animals were observed for toxic signs before, during, and at the end of each day's exposure. Animal body weights were recorded prior to the first inhalation exposure and on Monday of weeks 3, 5, 7, 9, 11, 13 and 3 days post exposure.

(2) Dogs were given a complete physical examination pretest and at weekly intervals. Pulmonary function measurements (compliance and resistance) were determined on each of the dogs prior to the test and at the termination of exposure. Dogs were anesthetized with Surital® (thiamylal sodium), 4 percent by intravenous injection through a pediatric catheter secured in the cephalic vein. A cuffed endotracheal tube was inserted in the trachea and the distal end connected to a Fleisch pneumotach for the measurement of airflow. Intrapleural pressure was measured by means of an esophageal balloon. Transpulmonary pressure (the difference between esophageal pressure and airway pressure derived from a lateral tap at the distal end of the endotracheal tube) was used for all calculations. Both flow and transpulmonary pressure were recorded with Statham Model PM 15 differential pressure transducers. Flow and pressure signals were processed in a Buxco Electronics, Inc. Pulmonary Function Computer, and the following parameters were recorded on a Honeywell, Fiber Optics Recorder (Model 1858): flow, tidal volume, transpulmonary pressure, compliance, and resistance. These parameters were measured or computed dynamically on a breath-to-breath basis and averaged every fifth breath.

(3) Groups of male and female rats were monitored for oxygen consumption as a means of estimating changes in the overall metabolic state of animals inhaling the m-Det aerosol. Rats in groups of five were tested in 15-minute periods, 2 days per week (Monday morning and Friday afternoon), prior to and after corresponding exposures. A water-sealed, 22-liter, plexiglass chamber connected to a 1-liter Collins Spirometer was used to test oxygen consumption. The total oxygen consumed expressed as liters/kg/day was calculated by dividing the volume of oxygen used by total body weight and projected to a 24-hour period. Exhaled CO_2 was eliminated continuously by cycling the chamber air through a container of Baralyme®.

® Surital is a registered trademark of Park Davis & Co., Detroit, MI 48232.
® Baralyme is registered trademark of Chemetron Medical Products Division, Chemetron Corp, St Louis, MO 53110.

(4) The clinical laboratory measurements listed in Table 2 were made on all dogs. Five pretest baseline and five exposure period values were determined.

TABLE 2. CLINICAL LABORATORY MEASUREMENTS

Hematology	
Total Erythrocyte Count	Hemoglobin
Total Leukocyte Count	Hematocrit
Differential Leukocyte Count	Mean Corpuscular Volume
Blood Chemistry	
Red Blood Cell and Plasma Cholinesterase	
Triglycerides	
Gamma Glutamyl Transpeptidase (GGTP)	
Blood Urea Nitrogen (BUN)	
Total Protein	
Serum Alkaline Phosphatase	
Total Bilirubin	
Glucose	
Serum Lactic Dehydrogenase (LDH)	
Cholesterol	
Serum Glutamic Pyruvic Transaminase (SGPT)	
Serum Glutamic Oxaloacetic Transaminase (SGOT)	
Sodium (Na)	
Potassium (K)	
Calcium (Ca)	

The preceding determinations were also made on groups of five rats of each sex from each concentration level prior to exposure, at the beginning of week 7, and at termination of the testing period. Gross necropsies were performed on all remaining animals at the end of the 13-week exposure. The rat liver, kidney, brain, heart, and testes weights were recorded. Sample from each tissue listed in Table 3 were collected from each animal, preserved in 10-percent neutral formalin, and examined for histopathologic lesions.

Study No. 75-51-0034-80, Jan-May 79

TABLE 3. TISSUE SAMPLES COLLECTED DURING NECROPSY

any unusual lesions	kidney
brain (cerebrum, cerebellum, brainstem)	skin
spinal cord	skeletal muscle
eye	uteri
pituitary gland	gall bladder
salivary gland	trachea
heart	spleen
thymus	femur (with marrow)
	sternum (with marrow)
lung	bronchi
esophagus	pancreas
thyroid	liver
adrenal glands	aorta
stomach	urinary bladder
small intestine (duodenum, ileum, jejunum)	nasal passages
large intestine (colon, cecum)	paranasal sinuses
ovaries/testes	prostate
mammary glands	nares

Animal body weights, organ-to-body weight ratios, blood chemistry and hematology values, and all similar data were analyzed statistically.

(5) Spermhead morphology in rats was investigated* due to evidence of gonadotoxic effects of m-Det reported in the literature (reference 3, Appendix A). "The caudae epididymides were dissected and placed in centrifuge tubes (one per animal) that contained approximately 5 ml of 0.9 percent saline solution. The tubes were then transferred to Litton Bionetics' facilities in Kensington, MD, where the contents were transferred to petri dishes and cut into several small pieces with scissors. The resulting suspension was gently pipetted five or six times back and forth in a 5 or 10 ml pipette. The sperm solution was filtered through an 80-u silk mesh to remove tissue fragments, and 0.5 ml was transferred to a centrifuge tube to which 0.05 ml 1 percent Eosin 1 was added. The solution was gently agitated, and slides were prepared by placing one drop of the stained solution on a slide and spread by three passes of another slide. The slides were air-dried and mounted with Permount. One thousand sperm were examined per animal at 400X magnification. The number of sperm with clearly abnormal head morphologies was recorded. All slides were scored blind by one individual."

---

* The spermhead morphology study was performed by Litton Bionetics, in conjunction with this Agency, not as a definitive study but to set the groundwork for future investigations with m-Det in this area. These data have not been completely analyzed and results are not meant to be conclusive (Appendix O).

(6) The toxicity evaluation of m-Det was augmented with concurrent behavioral evaluations carried out at intervals during the 13-week exposure period with rats already participating in the subchronic inhalation study. The behavioral evaluation procedures are described in a separate report (reference 4, Appendix A).

7. EXPOSURE CONCENTRATIONS AND ANALYSIS.

a. Saturated Vapor. The nominal exposure concentrations for the saturated vapor tests were based on the weight loss of m-Det during the 8-hour exposure period. No loss of compound was measured after the 8-hour 26°C exposure. The nominal concentration for the 100°C exposure was 2.5 mg/L.

b. Acute Aerosol Exposures. The concentrations for the acute aerosol exposures were measured analytically (see paragraph 6d). Six 4-hour exposures were performed. The time-weighted average for these studies were 3700, 4260, 5185, 5450, 5480 and 5950 mg/m^3.

c. Subchronic Aerosol Exposures. The concentrations for the subchronic aerosol exposures were measured analytically (see paragraph 6d). Table 4 is a list of the weekly averages of 6-hour time-weighted averages.

TABLE 4. WEEKLY AVERAGES OF THE DAILY TIME-WEIGHTED AVERAGES

Week No.	High Exposure Chambers	Medium Exposure Chambers (mg/m^3)	Low Exposure Chambers (mg/m^3)
1	1501	800	263
2	1430	777	286
3	1481	754	253
4	1490	758	218
5	1420	780	243
6	1485	682	220
7	1537	732	226
8	1495	806	228
9	1614	742	227
10	1544	717	250
11	1488	771	260
12	1558	801	269
13	1597	760	294

The mean and standard deviations of the daily time-weighted averages for the subchronic aerosol exposures are listed in Table 5.

TABLE 5. DAILY TIME WEIGHTED AVERAGES FOR SUBCHRONIC AEROSOL STUDY

High Exposure	Medium Exposure	Low Exposure
1511 mg/m^3 ±165	752 mg/m^3 ±80	253 mg/m^3 ±44

d. Particle Size Distribution. Examination of aerosol particles for Count Median Diameter determination was impossible due to the evaporation of the particles on the slide before counting procedures could be completed.

8. SUMMARY OF ANIMAL TEST RESULTS.

a. General Saturated Vapor. No toxic signs were seen in the rats exposed to saturated vapor of m-Det at room temperature for the 8-hour exposure period. Transient hyperemia was seen in rats 45-60 minutes into the 100°C m-Det saturated vapor exposure. All toxic signs in rats disappeared overnight following the 8-hour exposures (Appendices D and E).

b. General Acute Inhalation. Table 6 is a summary of the male and female rat survival record compiled during the acute inhalation exposures.

TABLE 6. RAT SURVIVAL RECORD DURING ACUTE INHALATION EXPOSURES

	Response		
Concentration (mg/m^3)	Male Rats # Dead/# Exposed	Female Rats # Dead/# Exposed	Combined Male and Female Rats # Dead/# Exposed
3700	1/10	1/10	2/20
4260	0/10	0/10	0/20
5185	2/10	1/10	3/20
5450	3/10	2/10	5/20
5480	2/10	4/10	6/20
5950	7/10	8/10	15/20

This information was used to calculate the m-Det LC_{50} for rats (5950 mg/m^3). Appendices F and G are summaries of the acute aerosol LC_{50} data.

c. Body and Organ-to-Body Weight Ratio. The mean body weight of male and female rats of all groups during the saturated vapor, acute, and subchronic aerosol study periods are presented in Appendices D through N. No

exposure-related significant differences were observed in female and male body weights between control and exposed groups of animals during the saturated vapor or subchronic aerosol study periods, and over the postexposure periods. No significant differences in organ-to-body weight ratios were observed as a result of the saturated vapor exposures (Appendix E). Significant differences were found between the acute inhalation exposure rat and control rat body weights over the 14-day observation period. A very slight significant difference was observed in testes organ-to-body weight ratios of exposed compared to control rats necropsied 7 weeks into the 13 week aerosol exposure (Appendix K). This was not observed in the rats necropsed after 13 weeks of exposure (Appendix M). A significant difference was observed in liver organ-to-body weight ratios of exposed compared to control female rat necropsied 7 weeks into the subchronic exposure (Appendix L). A significant difference was also observed after 13 weeks in exposed male kidney and exposed male and female liver organ-to-body weight ratios when compared to control rats (Appendicies M and N). A repeated measure, analysis of variance, was used to evaluate differences in weight between groups and over time. Differences were considered significant at the 0.05 level.

d. Histopathology.* No gross lesions attributable to the inhalation of the insect repellent, m-Det, were detected in rats necropsied 14 days after the saturated vapor, acute aerosol, and immediately after the subchronic aerosol exposures. No chemically-induced histological lesions were recognized in the tissues microscopically examined (see paragraph 6e).

e. Dog CBC Parameters. All CBC parameters measured (see paragraph 6e) for all dogs except dog number 66 were within expected normal ranges. No trends in exposed versus control dogs, or pretest versus post test values were observed. Dog number 66 (low chamber female) had a relative neutropenia and a marked eosinophilia in the fifth week's sample.

f. Dog Blood Chemistry. All blood chemistry parameters measured (see paragraph 6e) except dog number 63 were within the expected normal range for young beagles. No trends in exposed versus controls or pretest versus post test were observed. Dog number 63 (control female) had a markedly elevated SGPT value in blood drawn 11 weeks into the subchronic exposure. All other SGPT measurements for this animal were normal.

g. Rat CBC and Blood Chemistry. No trends or clinically significant changes in any of the rat blood chemistry or CBC values were apparent after comparing exposed rat data to pretest and control data.

h. Spermhead Assay. The percentages of abnormal spermheads for each of the four treatment groups in the subchronic inhalation study are presented in Table 7. There are consistently higher frequencies of abnormal spermheads in the exposed animals, but only the mean of the high-dose group differs

---

* Tissue examination performed by Experimental Pathology Laboratories, Inc., Herndon, VA, August 1979, under Contract No. DAAD05-79-F-6364.

significantly from the control value (t_s = 2.162, df = 18, and P<0.05). One frequency in the control group and one in the low-dose group exceeded the group mean by >2 standard deviations, while the middle and high-dose frequencies were somewhat more tightly clustered around their respective means.*

TABLE 7. PERCENTAGE OF SPERM WITH ABNORMAL MORPHOLOGY IN RATS EXPOSED TO m-Det*

Negative Control (No.'s 261-270)	250 mg/m^3 (No.'s 321-330)	750 mg/m^3 (No.'s 301-310)	1500 mg/m^3 (No.'s 281-290)
1.2	0.9	6.5	8.1
1.7	5.9	3.8	2.3
2.8	17.4†	6.4	2.5
2.4	3.0	2.1	8.1
3.4	2.4	9.9	6.2
2.1	4.4	7.7	4.5
3.1	4.2	2.3	2.4
2.1	2.5	4.2	5.9
2.8	3.2	0.6	9.3
9.8†	5.9	4.2	6.4
Mean ±S: 3.4 ±2.3	4.9 ±4.4	4.7 ±2.7	5.5 ±2.4

* 1000 sperm were scored from each animal.
† Percentages exceeding the group mean +2 standard deviations.

1. Oxygen Consumption. The mean O_2 consumptions for each treatment group of male and female rats during the 13-week subchronic inhalation exposures are given in Table 8.

* Work performed by Litton Bionetics, Kensington, MD [Evaluation of Deet (N,N, Diethyltoluamide) in the Spermhead Abnormal Assay, 25 May 1979, Appendix O].

TABLE 8. COMPARISON OF OXYGEN CONSUMPTION IN RATS

Treatment Groups	Oxygen Consumption ml/g/day Mean ±SD Male	Female
Control	52 ± 19	65 ± 16
250 mg/m^3	61 ± 16	68 ± 13
750 mg/m^3	60 ± 13	69 ± 11
1500 mg/m^3	57 ± 14	69 ± 13

Comparison of the grouped data indicates that a, 6 hour per day, 13-week exposure to m-Det at 250, 750, and 1500 mg/m^3 did not produce a significant change in oxygen consumption.

j. Compliance and Resistance. Compliance and resistance functions of lungs were determined in all groups of dogs. Table 9 shows the pretest and post test values for compliance and resistance in treated and control dogs. The mean values in all groups of dogs were not different from pretest control values and were within reported normal limits (reference 5, Appendix A).

k. Results of Behavioral Tests. The behavioral evaluation screen was able to detect differences between all concentrations in the acute study and, after the sixth week of exposure, all concentrations in the subchronic study. No single test was able to differentiate between all concentrations for both sexes. Detailed results are reported separately (references 2 and 4, Appendix A).

l. Personnel. See Appendix P for a listing of personnel.

9. DISCUSSION.

a. The lack of chemically-induced lesions in tissues and organs of rats exposed to saturated vapor of m-Det and necropsied 14 days postexposure indicated no apparent potential for causing morphologic changes in tissues and organs of exposed rats. Ambrose in 1959 (reference 1, Appendix A) also reported no systemic toxic reaction in rats exposed 8 hours a day, 5 days a week, for 7 weeks to air saturated with m-Det.

b. Ambrose (reference 1, Appendix A) observed only slight toxic effects in rats exposed for 6 hours to an aerosol of m-Det. He also found no gross significant microscopic changes in those exposed rats. The only sign of toxic effects in his study, which was not always present, was a slight bloody discharge about the nares and eyes. These and other toxic signs appeared in

TABLE 9. PULMONARY FUNCTION STUDIES - COMPLIANCE AND RESISTANCE IN EXPOSED DOGS
(2 males & 2 females per exposure group)

Treatment Group	Pretest Compliance ml/cm H_2O/kg Mean + SD	Pretest Resistance cm H_2O/ml/sec/kg Mean & Range x 10^{-3}	Post Test Compliance ml/cm H_2O/kg Mean + SD	Post Test Resistance cm H_2O/ml/sec/kg Mean & Range x 10^{-3}
Controls	4.57 ± 1.80	1.35 ± 0.65	4.37 ± 1.80	1.05 ± 0.65
250 mg/m^3	5.30 ± 1.10	0.34 ± 0.07	5.25 ± 1.99	0.73 ± 0.20
750 mg/m^3	4.99 ± 0.91	0.62 ± 0.19	4.87 ± 2.87	1.26 ± 0.57
1500 mg/m^3	6.21 ± 3.29	1.09 ± 0.46	5.05 ± 1.45	1.08 ± 0.20

rats exposed to aerosols during the acute LC_{50} exposures in this study (Appendices F and G). The rat exposure concentrations are not given in the Ambrose study, so direct comparisons are impossible.

c. Transient toxic signs were seen in the rats and dogs exposed to m-Det during the 13-week subchronic inhalation study. Toxic signs ranged from red exudate around the eyes and nose of the rats exposed to 1500 mg/m^3 of m-Det to periodic vomiting of dogs exposed to 750 and 1500 mg/m^3 of the test compound. None of these signs appeared in any of the control dogs or rats.

d. Mean compliance and resistance values obtained from preexposure and postexposure dog lung measurements were not significantly different at the 0.05 level of significance. The lack of variation from the control measurements suggests that no lung irritation was produced in the dogs subjected to the 13-week subchronic inhalation study.

e. Comparison of the grouped rat oxygen consumption data indicates that a 13-week exposure to 250, 750 or 1500 mg/m^3 of m-Det produced no significant increase or decrease in oxygen consumption. These findings indicate that during the subchronic inhalation exposure there were no apparent alterations in general metabolism.

f. No gross lesions attributable to the inhalation of the m-Det aerosol were detected in rats necropsied 7 weeks into and the week following the 13-week subchronic study. No clinically-induced histological lesions were recognized in the tissue sections examined (see paragraph 6e) indicating no apparent potential for causing morphologic changes in tissues and organs of exposed rats.

g. In conjunction with the inhalation study, Litton Bionetics harvested rat sperm from exposed and control rats following the 13-week subchronic exposure. Their objective was to determine the effects of m-Det inhalation on spermhead morphology. They found it difficult to interpret the data because the actual increases in abnormal sperm frequency were quite small. They did state the following arguments for the conclusion that m-Det did induce sperm abnormalities. "First, the data suggest a positive dose response, as the highest frequency was observed at the high dose. Second, the t-test indicates significance at the 5-percent level. Third, the two unusually high frequencies (>mean + 2's) were observed in the control and low-dose groups, which tend to actually minimize, perhaps unrealistically, the difference between the control and higher-dose mean values. Fourth and finally, only one of 10 control animal frequencies exceeded even the lowest mean value observed in treated animals, while 7 of 10 animals in the high-dose group exceeded the mean observed in controls. Thus, it must be concluded that m-Det may have induced spermhead abnormalities under the

present conditions although the observed effects were small."* These findings, although indicating possibly induced sperm abnormalities, do not conform with the Russian study (reference 3, Appendix A) which indicates a two-to-five-fold difference in the number of abnormal sperm in dermally exposed male rats.

h. The concentrations of m-Det employed for the subchronic inhalation study can be related to the expected usage concentrations as follows. A typical 12-ounce pressurized spray can (as described in Federal Specification O-1-503) contains 75 percent diethyltoluamide (approximately 71 percent m-Det). A 10-second spray from an aerosol can (e.g., NSN 6840-00-720-9768) of m-Det would expose the occupants of a small 10-cubic meter room to about 700 mg per cubic meter, assuming the spray can emits about 1 gram per second. This is similar to the medium dose (750 mg/m^3) of our subchronic inhalation study, but exposure would be for seconds rather than weeks. If m-Det produces its major effect through skin penetration rather than inhalation, the levels would depend upon how much is actually applied and how often applications are repeated. A plastic container of repellent (e.g., NSN 6840-00-753-4963) calls for the use of about 2 ounces per day per person for some applications. This is actually far less than is likely to be squirted into the hand, when applying m-Det from this type of squeeze bottle. The frequency of repetition is not specified. According to a report by Feldmann and Maibach (reference 6, Appendix A), m-Det is absorbed through the skin at approximately 0.75 percent of the applied dose per hour up to a total absorption of 20 percent of the applied dose in man. It is quite possible that sufficient m-Det could be applied to equal the exposures used in our test.

10. CONCLUSIONS. It is concluded that inhalation of m-Det insect repellent at the 750-mg/m^3 level and below presents little acute inhalation hazard to man. Concentrations above this level may cause transitory eye and respiratory irritation. M-Det may induce spermhead abnormalities at the high concentration (1500 mg/m^3) tested, although the observed effects were small. Behavioral changes were evidenced at all exposure levels, but their significance to human health is unknown.

---

* Work performed by Litton Bionetics, Kensington, MD [Evaluation of Deet (N-N, Diethyltoluamide) in the Spermhead Abnormal Assay, 25 May 1979, Appendix O].

Study No. 75-51-0034-80, Jan-May 79

11. RECOMMENDATION. The insect repellent, m-Det, does not appear to present an acute toxic inhalation hazard to man at the expected usage levels. It is, therefore, recommended that approval be granted for the use of m-Det spray insect repellent with some consideration for ventilation of any area where extended spray application procedures take place.

JOSEPH A. MACKO, JR.
Biologist
Toxicology Division

for JEFFREY D. BERGMANN
SP4, USA
Veterinary Specialist
Toxicology Division

APPROVED:

ARTHUR H. McCREESH, Ph.D.
Chief, Toxicology Division

APPENDIX A

REFERENCES

1. Pharmacologic and Toxicologic Studies on N,N-Diethyltoluamide (N,N-Diethyl-m-Toluamide), Anthony M. Ambrose, Toxicology and Applied Pharmacology, 1, 97-115 (1959)

2. Report, HSE-LT/WP, this Agency, Toxicological Assessment Program Special Study No. 75-51-0034-79, Behavioral Effects of Acute Aerosol Exposure to N, N-Diethyl-Meta-Toluamide (M-Det), January - February 1979.

3. Experimental Study of the Long-Term Effects of Using Repellents. Report I. An Experimental Study of the Effects of Long-Term Use of Diethyl-Toluamide (DETA) Repellent, S. Ye. Gbyberman, A. P. Volkova, G. M. Nikolayer and Ye. V. Lhukova.

4. Report, HSE-LT/WP, this Agency, Behavioral Effects of Subchronic Aerosol Exposure to m-Det, Study No. 75-51-0034-79, February - May 1979.

5. Altman, P. L. and D. S. Dittner, Eds.; Respiration and Circulation, Biological Handbook Series, p 95, Federation of American Societies for Experimental Biology, Bethesda, MD (1971).

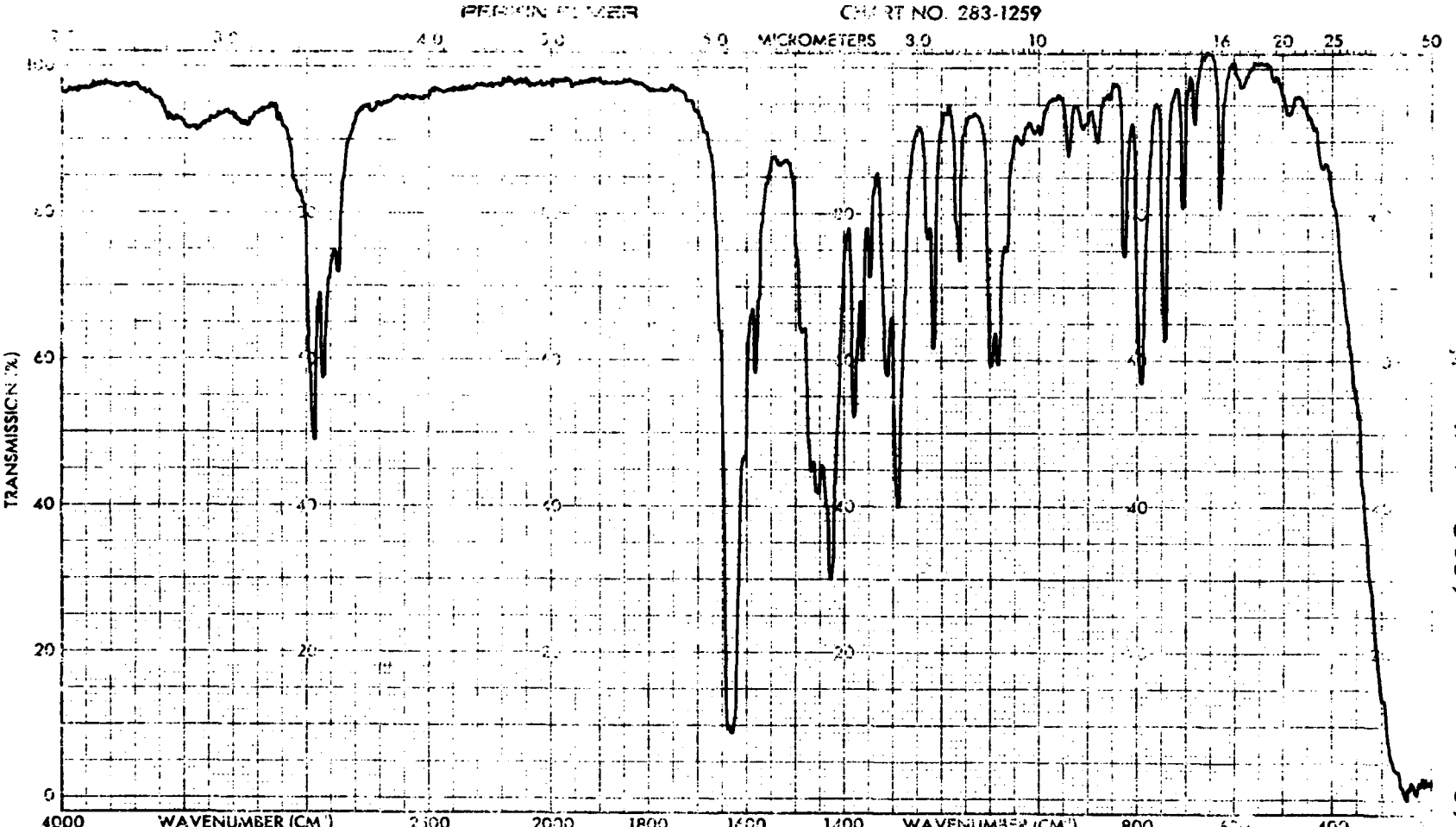
6. Absorption of Some Organic Compounds Through the Skin in Man, Robert J. Feldmann, M.D., and Howard I. Maibach, M.D., Journal of Investigative Dermatology, Vol. 51, No. 5, 1970.

7. Toxicological Division Procedural Guide, USAEHA, 1972, revised 1976.

Study No. 75-51-0034-80, Jan-May 79

APPENDIX B

INFRARED SPECTRUM OF N,N-DIETHYL-META-TOLUAMIDE



ABSCISSA	ORDINATE		
EXPANSION 1	EXPANSION 1	SCAN TIME 6 min	REP. SCAN — SINGLE BEAM —
SUPPRESSION ✓	% T 0 100% ABS —	RESPONSE 1	TIME DRIVE — PRE SAMPLE CHOP —
		SLIT PROGRAM 6	OPERATOR McKenzie DATE 5 June 79
SAMPLE ACGSB# 5657 N,N-Diethyl-m-Toluamine	REMARKS Tox 51-0034-28	SOLVENT —	CELL PATH Cap film KBr
ORIGIN Weeks-Tox		CONCENTRATION Neat	REFERENCE Air

B-2

ACGSB# 5657

5/25/79

APPENDIX C

GLOSSARY OF RECURRING DEFINITIONS, ABBREVIATIONS, AND SYMBOLS USED BY THE TOXICOLOGY DIVISION, USAEHA

Definitions of terms and abbreviations used in this report are in agreement with the Stedman's Medical Dictionary, 20th ed., published by the Williams and Wilkins Company. The following terms and abbreviations are either not found in the above reference or have been modified to fit the special purposes of this report. Some of the terms have been included below for special emphasis.

DEFINITIONS

Word	Definition
Acute Exposure	One exposure to exogenous test material for no longer than 8 hours.
Anova	Analysis of Variance
Count Median Diameter	The value of the diameter of a particle analysis for which the vertical coordinates divides the area of a percentage distribution curve into halves with respect to the surface.
Minute Volume	The total amount of new air exchanged in the lungs each minute, and this is equal to the tidal volume (volume of air inspired and expired with each normal breath) times the respiratory rate.
Nominal Concentration	Concentration of compound in the exposure chambers as determined by ascertaining the weight of the sample lost from the dispersion apparatus divided by total volume of chamber air used throughout the exposure time.
Pulmonary Compliance	Volume change produced in the lung by a unit pressure change.
Pulmonary Resistance	Pressure differential required for a unit flow change in the lung.
Subchronic Exposure	Repeated daily or constant exposure to a test material for no longer than 179 days or less than 2 days. Postobservation period will vary.

Tidal Volume	The volume of air inspired and expired with each normal breath.

ABBREVIATIONS

Abbreviation	Meaning
cmd	Count Median Diameter
gm	gram
l or L	liter of air
mg	milligram
lpm or Lpm	liters per minute

SYMBOLS

Symbol	Meaning
>	greater than
<	less than

Study No. 75-51-0034-80, Jan-May 79

APPENDIX D
SATURATED VAPOR INHALATION EXPOSURE
8-HR EXPOSURE OF MALE RATS TO N,N-DIETHYL-M-TOLUAMIDE

	Mean Body Weights (gm)				
Exposure Groups	Preexposure	Post Exposure Day 1	Post Exposure Day 3	Post Exposure Day 7	Post Exposure Day 14
Room Air Control	129	133	145	162	217
Sat Vapor (m-Det) 26°C	132	135	150	172	232
Sat Vapor (m-Det) 100°C	136	138	153	177	221
F Value (Anova)	.9771	.6689	1.2639	2.9918	1.6525
Degree of Freedom	2,10	2,10	2,10	2,10	2,10

NOTE: No significant differences between control and exposed groups (p = 0.05).

Study No. 75-51-0034-80, Jan-May 79

APPENDIX E

SATURATED VAPOR INHALATION EXPOSURE
8-HR EXPOSURE OF MALE RATS TO N,N-DIETHYL-M-TOLUAMIDE

(Mean organ-to-body weight ratio
grams per 100 grams body weight)

Exposure Groups	Mean Terminal Body Weight (gm)	Lung	Liver	Spleen	Kidneys	Testes
Room Air Control	217	0.69	4.68	0.48	0.86	0.98
Sat Vapor (m-Det) 26°C	231	0.64	4.85	0.43	0.91	0.99
Sat Vapor (m-Det) 100°C	221	0.57	4.50	0.40	0.80	1.01
F Value (Anova)	1.6525	3.1764	1.3873	2.6584	1.5104	.2298
Degree of Freedom	2,10	2,10	2,10	2,10	2,10	2,10

NOTE: No significant differences between control and exposed groups (p = 0.05).

APPENDIX F

COMPOUND: M-DET — USAEHA STUDY NO. 75-51-0034-80

ACUTE AEROSOL LC50
MALE RATS
SPRAGUE-DAWLEY,

LC50 6047 mg/m³ — 95% C.L. 4958 - 7376 mg/m³

Slope 9.44 — S.E. 3.63

Conditions N,N-diethyl-m-toluamide minimum meta isomer 95% & 5% otherisomers.

DATE	Conc mg/m³	Onset of signs (s), mortality (m) — Hours: 0-4	4-12	12-24	Days: 2	3	4	5	6	7	8-14	Mort Cumulative	Mean Body Wt. Init	Mean Body Wt. Fin	Mean Body Wts. (g) Days 1	3	7	14
	3700		10(S^{5+2})	1(S^{4+4}) 3(S^{5}) 1(m)								1/10	119 ± 7	219 ±12	110 ±10	132 ± 9	165 ±10	219 ±12
	CONTROL											0/10	120 ± 7	240 ±16	126 ± 7	145 ± 8	176 ±12	240 ±16
	4260											0/10	126 ± 5	236 ± 9	115 ± 8	137 ± 8	172 ± 8	236 ± 9
	CONTROL											0/10	98 ±5	211 ±13	106 ± 5	114 ±11	153 ± 8	211 ±13
	5185		10(s^{1+2})	1(m)	1(m)							2/10	93 ±9	203 ±15	82 ±8	99 ±13	131 ±13	203 ±15
	CONTROL											0/10	93 ±9	215 ±11	100 ±10	116 ±12	146 ±11	215 ±11
	5450		10(s^{1+2})	1(m)	2(m)							3/10	132 ±10	201 ±14	118 ±10	124 ±15	160 ±14	201 ±14
	CONTROL											0/10	134 ± 7	230 ±31	145 ± 8	153 ±10	193 ±13	230 ±31
	5480		10(s^{1+2})	2(m)								2/10	101 ± 7	205 ±17	87 ±5	95 ±14	132 ±13	205 ±17
	CONTROL											0/10	100 ±10	215 ±11	107 ±10	123 ±10	153 ±10	215 ±11
	5950		2(S^{3}) 10(s^{1+2})	1(S^{3}) 8(S^{1+2+4}) 2(m)	1(S^{4}) 2(S^{5}) 4(S^{7}) 4(m)	1(m)						7/10	124 ±14	222 ±23	108 ±10	119 ±20	160 ±20	222 ±23
	CONTROL											0/10	130 ±14	245 ±21	125 ±40	158 ±16	189 ±16	245 ±21

Signs of Intoxication 1) Unconscious 2) Labored breathing 3) Slight convulson 4) Nasal discharge 5) Ataxia 6) Red exodate around nose 7) Ruffled hair 8) Tremors 9) Red urine

Gross Autopsy: Decedents - No gross lesions; Survivors - No gross lesions

APPENDIX G

COMPOUND: USAEHA STUDY NO. 75-51-0034-80

ACUTE AEROSOL LC50
FEMALE RATS
SPRAGUE-DAWLEY,

LC50 5862 mg/m³ 95% C.L. 4805 - 7151 mg/m³
Slope 10.99 S.E. ± 3.93
Conditions N,N-diethyl-m-toluamide minimum meta isomer 95% & 5% other isomers.

DATE	Conc mg/m³	Onset of signs (s), mortality (m) Hours 0-4	Hours 4-12	Hours 12-24	Days 2	Days 3	Days 4	Days 5	Days 6	Days 7	Days 8-14	Mort Cumulative	Mean Body Wt. Init	Mean Body Wt. Fin	Mean Body Wts. (g) Days 1	Days 3	Days 7	Days 14
	3700		10(S⁵⁺²)	1 (S⁵) 2 (S⁵) 1 (m)								1/10	102 ± 6	168 ± 8	97 +8	112 +12	136 +10	168 ± 8
	CHAMBER CONTROL											0/10	107 +11	178 +15	108 +11	123 +12	143 +11	178 +15
	4260											0/10	102 ± 8	188 +15	96 +14	114 +11	143 +10	188 +15
	CHAMBER CONTROL											0/10	98 +6	179 +11	104 ± 6	117 ± 6	144 ± 7	179 +11
	5185		10(S¹⁺²)		1(m)							1/10	85 ±8	160 +11	76 +9	84 +10	113 +10	160 +13
	CHAMBER CONTROL											0/10	85 +9	164 +11	91 +9	103 +10	126 +11	164 +11
	5450		10(S¹⁺²)	1 (m)	1(m)							2/10	117 ± 8	172 +10	109 ± 9	110 +10	139 +14	172 ± 9
	CHAMBER CONTROL											0/10	116 +11	178 +15	124 +12	126 +11	154 +11	178 +15
	5480		10(S¹⁺²)	2(m)	2(m)							4/10	91 +7	170 +11	76 +3	90 +3	118 ± 2	170 +11
	CHAMBER CONTROL											0/10	91 +9	164 +11	95 +9	107 ± 9	131 +11	164 +11
	5950		1 (S³) 10(S¹⁺²)	1 (S³) 8 (S¹⁺²⁺⁴) 2(m)	1 (S⁶) 2 (S⁵⁺⁷) 6(m)							8/10	118 +10	168 ± 8	106 +10	96 +13	128 ± 6	168 ± 8
	CHAMBER CONTROL											0/10	109 ± 6	174 +12	109 +12	129 ± 9	148 +10	174 +12

Signs of Intoxication 1) Unconscious 2) Labored breathing 3) Slight convulsions 4) Nasal discharge 5) Ataxia 6) Red exudate around nose 7) Ruffled hair 8) Tremors 9) Red urine

Gross Autopsy: Decedents - No gross lesions; Survivors - No gross lesions

Study No. 75-51-0034-80, Jan-May 79

APPENDIX H

SUBCHRONIC INHALATION AEROSOL EXPOSURES
6 HOUR, 5 DAY, 13 WEEK EXPOSURE OF MALE RATS TO
N,N-DIETHYL-M-TOLUAMIDE

	Mean Body Weights (gm)							
Chamber Conc Groups	Pre-exposure Day 1	Exposure Week +3	Exposure Week 5	Exposure Week 7	Exposure Week 9	Exposure Week 11	Exposure Week 13	Post Exposure Day +3
Chamber Control	131	231	325	377	421	461	488	493
1500 mg/m^3	128	217	305	349	394	426	458	461
750 mg/m^3	127	225	312	366	407	438	465	477
250 mg/m^3	131	230	316	359	413	447	474	472
F Value (Anova)	0.4351	1.1804	1.990	1.7143	1.6223	2.0159	1.2274	1.1768
Degrees of Freedom	3.42	3.42	3.42	3.27	3.27	3.27	3.27	3.27

NOTE: No significant differences between control and exposed groups (p = 0.05).

Study No. 75-51-0034-80, Jan-May 79

APPENDIX I
SUBCHRONIC INHALATION AEROSOL EXPOSURES
6 HOUR, 5 DAY, 13 WEEK EXPOSURE OF FEMALE RATS TO
N,N-DIETHYL-M-TOLUAMIDE

	Mean Body Weight (gm)							
Chamber Conc Groups	Pre-exposure Day 1	Exposure Week +3	Exposure Week +5	Exposure Week +7	Exposure Week +9	Exposure Week +11	Exposure Week +13	Post Exposure Day +3
Chamber Control	112	173	215	234	257	267	285	286
1500 mg/m^3	104	170	216	244	271	285	301	300
750 mg/m^3	108	170	215	239	283	268	284	285
250 mg/m^3	118	176	216	247	273	279	296	298
F Value (Anova)	4.1602*	0.8475	0.0290	2.1688	2.4306	3.0265*	2.7592	2.3912
Degrees of Freedom	3,39	3,39	3,39	3,27	3,27	3,27	3,27	3,27

* Significant at p = 0.05. No other statistical differences observed.

APPENDIX J

SUBCHRONIC INHALATION AEROSOL EXPOSURE
6 HOUR, 5 DAY, 13 WEEK EXPOSURE OF MALE AND FEMALE DOGS TO
N,N-DIETHYL-M-TOLUAMIDE

Chamber Conc.	Sex	No.	Tatoo No.	Mean Body Weight (kg) -10 Weeks	-8 Weeks	-6 Weeks	-4 Weeks	-1 Week	+2 Weeks	+5 Weeks	+8 Weeks	+11 Weeks	+13 Weeks
Control	♂	53	GK87	12.2	13.5	13.5	13.8	13.8	13.0	13.0	13.4	13.3	13.8
Control	♂	54	OQ87	12.8	13.7	13.6	13.2	13.4	14.0	14.2	15.2	14.8	15.8
Control	♀	63	EO87	9.0	9.7	10.0	10.0	9.6	10.0	9.4	10.2	9.2	9.8
Control	♀	64	TQ87	14.0	15.1	14.5	14.1	14.4	13.5	13.2	13.0	13.1	14.0
				12.0	13.0	12.9	12.8	12.8	12.6	12.5	13.0	12.6	13.4
1500 mg/m^3	♂	47	QP87	14.8	15.0	14.8	14.7	15.6	15.1	15.2	15.5	15.7	16.4
1500 mg/m^3	♂	48	RQ87	14.4	15.0	14.2	14.0	14.6	14.2	14.2	15.2	15.7	15.8
1500 mg/m^3	♀	69	RR87	10.0	10.6	10.4	9.6	10.0	9.2	10.0	10.6	10.5	10.9
1500 mg/m^3	♀	70	TV87	9.7	10.0	10.8	10.8	10.5	10.6	10.4	10.8	10.9	11.2
				12.2	12.7	12.6	12.3	12.7	12.3	12.5	13.0	13.2	13.6
750 mg/m^3	♂	49	BO87	10.4	11.0	11.0	10.4	11.5	11.4	11.5	12.4	12.1	11.8
750 mg/m^3	♂	50	XK87	13.2	13.8	12.6	12.1	13.7	13.5	13.6	14.6	13.9	14.4
750 mg/m^3	♀	67	YQ87	10.0	10.6	10.4	10.6	10.8	10.7	11.0	11.2	11.7	11.8
750 mg/m^3	♀	68	BD87	9.4	10.1	10.4	10.0	10.1	10.8	11.0	11.1	11.2	10.8
				10.8	11.4	11.1	10.8	11.5	11.6	11.8	12.3	12.2	12.2
250 mg/m^3	♂	51	GP87	11.0	11.8	12.0	11.8	12.5	11.8	12.8	13.0	13.2	13.6
250 mg/m^3	♂	52	GB87	13.0	13.5	14.0	13.8	13.6	14.0	15.2	14.2	14.8	15.5
250 mg/m^3	♀	65	QO87	12.4	12.5	12.5	11.8	12.4	12.8	12.6	13.0	12.8	13.1
250 mg/m^3	♀	66	YP87	11.5	12.0	11.8	11.4	11.6	12.0	12.2	12.2	12.2	12.6
				11.1	12.4	12.6	12.2	12.5	12.7	13.9	13.1	13.3	13.7
F Value (Anova)				.5887	.6718	1.4332	1.5762	.5518	.4741	.8205	.3686	.5550	.9094
Degrees of Freedom				3,9	3,9	3,9	3,9	3,9	3,9	3,9	3,9	3,9	3,9

NOTE: No significant differences between control and exposed groups (p = 0.05).

Study No. 75-51-0034-80, Jan-May 79

APPENDIX K

SUBCHRONIC INHALATION AEROSOL EXPOSURE
6 HOUR, 5 DAY, 7 WEEK EXPOSURE OF MALE RATS TO
N,N-DIETHYL-M-TOLUAMIDE

Chamber Conc Groups	Mean Terminal Body Wgt (gm)	Mean Organ-To-Body Weight Ratio Grams Per 100 Grams Body Weight					
		Liver	Kidney	Brain	Heart	Testes 1	Testes 2
Chamber Control	408.6	3.87	.70	0.51	0.34	0.38	0.39
1500 mg/m^3	370.6	4.40	.95	0.59	0.42	0.48	0.46
750 mg/m^3	386.2	3.84	.65	0.57	0.34	0.43	0.44
250 mg/m^3	396.0	3.97	.65	0.53	0.25	0.44	0.44
F Value (Anova)	0.7585	1.9086	1.7922	0.8834	3.1131	3.8242*	1.5859
Degree of Freedom	3,12	3,12	3,12	3,12	3,12	3,12	3,12

* Significant at p = 0.05. No other statistical differences observed.

Study No. 75-51-0034-80, Jan-May 79

APPENDIX L

SUBCHRONIC INHALATION AEROSOL EXPOSURE
6 HOUR, 5 DAY, 7 WEEK EXPOSURE OF FEMALE RATS TO
N,N-DIETHYL-M-TOLUAMIDE

Chamber Conc Groups	Mean Terminal Body Weight (gm)	Mean Organ-To-Body Weight Ratio Grams Per 100 Grams Body Weight			
		Liver	Kidney	Brain	Heart
Chamber Control	221.0	2.9300	0.79	0.87	0.35
1500 mg/m^3	242.2	4.4593	0.80	0.81	0.38
750 mg/m^3	235.5	4.3360	0.80	0.82	0.35
250 mg/m^3	225.8	4.3855	0.83	0.88	0.37
F Value (Anova)	1.9236	23.9182*	0.7757	3.8153	0.5451
Degree of Freedom	3,9	3,9	3,9	3,9	3,9

* Significant at p = 0.05. No other statistical differences observed.

Study No. 75-51-0034-80, Jan-May 79

APPENDIX M
SUBCHRONIC INHALATION AEROSOL EXPOSURE
6 HOUR, 5 DAY, 13 WEEK EXPOSURE OF MALE RATS TO
N,N-DIETHYL-M-TOLUAMIDE

Chamber Conc Groups	Mean Terminal Body (gm)	Mean Organ-To-Body Weight Ratio Grams Per 100 Grams Body Weight					
		Liver	Kidney	Brain	Heart	Testes 1	Testes 2
Chamber Control	492.5	3.13	.66	.44	.33	.35	.37
1500 mg/m^3	461.1	3.61	.75	.46	.31	.37	.36
750 mg/m^3	476.9	3.53	.77	.46	.32	.37	.35
250 mg/m^3	471.2	3.39	.76	.45	.30	.37	.37
F Value (Anova)	1.1767	10.1586*	7.925*	.7433	.6178	.5195	.4291
Degree of Freedom	3,27	3,27	3,27	3,27	3,24	3,27	3,27

*** Significant at p = 0.05. No other statistical differences observed.**

Study No. 75-51-0034-80, Jan-May 79

APPENDIX N

SUBCHRONIC INHALATION AEROSOL EXPOSURE
6 HOUR, 5 DAY, 13 WEEK EXPOSURE OF FEMALE RATS TO
N,N-DIETHYL-M-TOLUAMIDE

Chamber Conc Groups	Mean Terminal Body (gm)	Mean Organ-To-Body Weight Ratio Grams Per 100 Grams Body Weight			
		Liver	Kidney	Brain	Heart
Chamber Control	286.1	3.33	.70	.72	.35
1500 mg/m^3	299.7	3.79	.73	.68	.34
750 mg/m^3	284.5	3.46	.71	.72	.34
250 mg/m^3	298.4	3.43	.72	.68	.34
F Value (Anova)	2.3912	6.8361*	1.3102	1.77	0.2866
Degree of Freedom	3,27	3,27	3,27	3,27	3,27

*** Significant at p = 0.05. No other statistical differences observed.**

Study No. 75-51-0034-80, Jan-May 79

APPENDIX O

EVALUATION OF DEET (N, N-DIETHYLTOLUAMIDE) IN THE SPERMHEAD ABNORMALITY ASSAY

FINAL REPORT

SUBMITTED TO

S. C. JOHNSON & SON, INC.
1525 HOWE STREET
RACINE, WI 53403

SUBMITTED BY

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

SEPTEMBER 1979

Study No. 75-51-0034-80, Jan-May 79

Spermhead Abnormality Assay in Rats Exposed to DEET (N,N-Diethyltoluamide)

Submitted by: Daniel Stetka, Ph.D.
Department of Genetics
and Cell Biology
Litton Bionetics, Inc.

INTRODUCTION

The objective of this study was to determine the effects of N,N-Diethyl-m-toluamide inhalation, if any, on spermhead morphology in rats. Sprague Dawley derived rats (100-125 gm) were exposed subchronically (by U.S. Army personnel at Aberdeen, Md.) for 6 hrs per day, 5 days per week for 13 weeks at 250, 750 and 1500 mg/m^3 and their sperm were then harvested by Litton Bionetics' personnel for subsequent analysis of morphology (see below). The animals had been assigned code numbers prior to Litton's involvement, and the code was not broken until the raw data had been gathered, tabulated and forwarded to Aberdeen. Four groups of 10 animals each were employed in this study, three exposure levels plus one negative control.

MATERIALS AND METHODS

Animals were killed in Aberdeen by decapitation. The caudae epididymides were dissected and placed in centrifuge tubes (one per animal) that contained approximately 5 ml of 0.9% saline solution. The tubes were then transferred to Litton facilities in Kensington, Md. where the contents were transferred to petri dishes and cut into several small pieces with scissors. The resulting suspension was gently pipetted five or six times back and forth in a 5 or 10 ml pipette. The sperm solution was filtered through an 80μ silk mesh to remove tissue fragments and 0.5 ml was transferred to a centrifuge tube to which 0.05 ml 1% Eosin Y was added. The solution was gently agitated, and slides were prepared by placing one drop of the stained solution on a slide and spreading by three passes of another slide. The slides were air-dried and mounted with Permount. One-thousand sperm were examined per animal at 400X magnification. The number of sperm with clearly abnormal morphologies was recorded. All slides were scored blind by one individual. Additional protocol details are given in U.S. Army Study No. 75-51-0034-79.

RESULTS

The percentages of abnormal sperm for each of the 4 treatment groups are presented in Table 1. There are consistently higher frequencies of abnormal sperm in the exposed animals, but only the mean of the high dose group differs significantly from the control value, according to the following 5-test of the difference between two means, $\bar{Y}_1$ and $\bar{Y}_2$:

$$t_s = \frac{\bar{Y}_1 - \bar{Y}_2}{\left(\frac{s_1^2 + s_2^2}{n} \right)^{1/2}},$$

where s = standard deviation (see Table 1), n = sample size (10), and df = 2(n - 1) = 18.

In this case (high dose vs control), $t_s = 2.162$ and $P < 0.05$. One frequency in the control group and one in the low dose group exceeded the group mean by > 2 standard deviations, while the middle and high dose frequencies were somewhat more tightly clustered around their respective means.

DISCUSSION

It is difficult to interpret these data because the actual increases in abnormal sperm frequency are quite small. This argues against attaching too much biological meaning to these results. Several arguments can be made, however, for the conclusion that DEET did induce sperm abnormalities. First, the data suggest a positive dose response, as the highest frequency was observed at the high dose. Second, the t-test indicates significance at the 5% level (although the validity of the test could be argued, e.g., a nonparametric test should perhaps be applied). Third, the two unusually high frequencies (> mean + Zs) were observed in the control and low dose groups, which tends to actually minimize, perhaps unrealistically, the difference between the control and higher dose mean values. Fourth, and finally, only one of 10 control animal frequencies exceeded even the lowest mean value observed in treated animals, while 7 of 10 animals in the high dose group exceeded the mean observed in controls. Thus, it must be concluded that DEET induced spermhead abnormalities under the present conditions, although the observed effects were small.

Study No. 75-51-0034-80, Jan-May 79

TABLE 1

PERCENTAGES OF SPERM WITH ABNORMAL MORPHOLOGY
IN RATS EXPOSED TO DEET+

Negative Control (Nos. 261-270)	250 mg/m^3 (Nos. 321-330)	750 mg/m^3 (Nos. 301-310)	1500 mg/m^3 (Nos. 281-290)
1.2	0.9	6.5	8.1
1.7	5.9	3.8	2.3
2.8	17.4*	6.4	2.5
2.4	3.0	2.1	8.1
3.4	2.4	9.9	6.2
2.1	4.4	7.7	4.5
3.1	4.2	2.3	2.4
2.1	2.5	4.2	5.9
2.8	3.2	0.6	9.3
9.8*	5.9	4.2	6.4
Mean ± s: 3.14 ± 2.31	4.98 ± 4.40	4.77 ± 2.70	5.57 ± 2.43

+1000 sperm were scored from each animal

* Percentages exceeding the group mean +2 standard deviations.

Code Nos. supplied by the sponsor following slide scoring.

Study No. 75-51-0034-80, Jan-May 79

Key Personnel

Daniel Stetka, Ph.D., Study Director

Helen Lebowitz, Technical Supervisor

Winesta Smith, Technician
Tom Cortina, Technician

STUDY INITIATION: APRIL 30, 1979

STUDY COMPLETION MAY 25, 1979

Study No. 75-51-0034-80, Jan-May 79

APPENDIX P

PERSONNEL

Study Director	M. Weeks, Toxicology Division (TOX), USAEHA
Project Leader	J. Macko, TOX
Chamber Exposure	J. Macko, J. Bergmann, TOX
Chamber Sampling	J. Macko, J. Bergmann, TOX
Analytical Procedure	K. Rybczinski, Environmental Chemistry Division
Analytical Chemistry	J. Bergmann, TOX
Clinical Chemistry	E. Haight, M. Michie, Radiological and Biological Chemistry Division
Behavior Studies	CPT R. Sherman, J. Kerby, A. Asaki, TOX
Quality Control	P. Sneeringer, Analytical Reference and Quality Assurance Division
Pathology	LTC C. Pope, TOX
Histology	H. Snodgrass, R. Rodriguez, D. Nelson, R. Angerhofer, TOX
Pulmonary Function Studies	R. Metker, CPT A. Singer, A. Asaki,
Animal Care and Procurement	CPT A. Singer, TOX
Animal Handling (Rats)	O. Choates, R. Rodriquez, TOX
Animal Handling (Dogs)	R. Arnold, L. Maxa, TOX